

What is claimed is:

- 1                   1.       A method of identifying a nucleotide in at least a first position in a  
2 polynucleotide sequence, comprising:  
3                   providing a polynucleotide target sequence;  
4                   hybridizing the target sequence with a first oligonucleotide probe, wherein:  
5                   the probe comprises a first subsequence of nucleotides, a first terminal nucleotide,  
6 and a first florescent label;  
7                   the subsequence is complementary to a portion of the target sequence that is  
8 immediately adjacent to the first position; and  
9                   the terminal nucleotide is complementary to one possible nucleotide in the first  
10 position;  
11                  contacting the hybridized probe and target sequence with polymerase extension  
12 reagents in a first extension reaction mixture;  
13                  monitoring a fluorescent signal from the first extension reaction mixture that is  
14 indicative of the presence or absence of polymerase extension of the probe, the presence of  
15 polymerase extension of the probe indicating that the terminal nucleotide is complementary to the  
16 nucleotide in the first position; and  
17                  identifying the nucleotide in the first position.  
18  
19                  2.       The method of claim 1, wherein the terminal nucleotide is a 3'-terminal  
20 nucleotide.  
21  
22                  3.       The method of claim 2, wherein the fluorescent label is coupled to the 3'-  
23 terminal nucleotide.  
24  
25                  4.       The method of claim 1, wherein the polymerase extension reagents include a  
26 3'-5' DNA polymerase enzyme.  
27  
28                  5.       The method of claim 1, wherein the first fluorescent label is coupled to the  
29 first terminal nucleotide.

1                   6.       The method of claim 1, wherein the probe is from about 10 to about 50  
2 nucleotides in length.

1                   7.       The method of claim 1, wherein the subsequence is from about 9 to about 49  
2 nucleotides in length.

1                   8.       The method of claim 1, wherein the polymerase extension reagents comprise  
2 a non-proofreading polymerase.

1                   9.       The method of claim 8, wherein the non-proofreading polymerase is selected  
2 from exonucleoase minus klenow fragment, Taq polymerase, and Thermosequanase.

1                   10.      The method of claim 1, wherein the contacting step occurs in a channel of a  
microfluidic device.

1                   11.      The method of claim 1, wherein the monitoring step comprises monitoring a  
2 level of polarized fluorescence emitted from the first extension reaction mixture, a decrease in  
3 polarized fluorescence indicating the presence of polymerase extension.

1                   12.      The method of claim 1, further comprising:  
2 hybridizing the target sequence with a second oligonucleotide probe that comprises:  
3 the first subsequence of nucleotides, a second terminal nucleotide, and a  
4 second florescent label, the second fluorescent label being distinguishable from the first fluorescent  
5 label; and

6                   the second terminal nucleotide is different from the first terminal nucleotide  
7 and is complementary to one possible nucleotide in the first position; and

8                   wherein the monitoring step comprises monitoring fluorescent signals from each of  
9 the first and second fluorescent labels, the fluorescent signal from one of the first and second  
10 fluorescent labels being indicative of polymerase extension of the first or second oligonucleotide  
11 probe, respectively.

1                   13.     A method for identifying a nucleotide in a first position in a target nucleic  
2 acid sequence, comprising:  
3                   amplifying the target nucleic acid sequence in a first reaction mixture that includes  
4 effective amounts of polymerase enzyme and four dNTPs;  
5                   introducing into the first reaction mixture a first primer sequence to produce a second  
6 reaction mixture under conditions conducive to a polymerase mediated primer extension, wherein  
7 the first primer sequence comprises a first subsequence of nucleotides, a first terminal nucleotide,  
8 and a first florescent label, wherein the subsequence is complementary to a portion of the target  
9 sequence that is immediately adjacent to the first position, and the first terminal nucleotide is  
10 complementary to one possible nucleotide in the first position;  
11                   monitoring a fluorescent signal from the second reaction mixture that is indicative of  
12 a presence or absence of extension of the first primer sequence; and  
13                   identifying the nucleotide in the first position based upon whether the fluorescent  
14 signal is indicative of the presence of extension of the first primer sequence.

1                   14.     A system for identifying at least a first nucleotide in a target nucleic acid  
2 sequence, comprising:  
3                   a reaction vessel having disposed therein:  
4                   a first target nucleic acid sequence having an unknown nucleotide at a first  
5 position;  
6                   a first oligonucleotide probe having a first subsequence of nucleotides, the  
7 first subsequence being complementary to a subsequence of nucleotides in the target sequence that  
8 are immediately adjacent to the first position, a first terminal nucleotide that is positioned to be  
9 adjacent to the first position when the first subsequence of the probe is hybridized to the  
10 subsequence of the target, and a first fluorescent label; and  
11                   polymerase extension reagents; and  
12                   a detector configured to monitor a fluorescent signal from the reaction vessel that is  
13 indicative of a presence or absence of polymerase extension of the probe.

1                   15.     The system of claim 14, wherein the first fluorescent label is coupled to the  
2 first terminal nucleotide.

1                    16.    The system of claim 14, wherein the first terminal nucleotide is a 3' terminal  
2 nucleotide, and the polymerase extension reagents include a 3'-5' DNA polymerase enzyme.

1                    17.    The system of claim 14, wherein the reaction vessel is selected from a  
2 reaction well in a multiwell plate, a capillary channel and a channel in a microfluidic channel  
3 network.

1                    18.    The system of claim 14, wherein the reaction vessel further comprises a  
2 second oligonucleotide probe disposed therein, the second oligonucleotide probe comprising the  
3 first subsequence of nucleotides, a second terminal nucleotide different from the first terminal  
4 nucleotide, that is complementary to one possible nucleotide in the first position, and a second  
5 fluorescent label.